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TECHNICAL MANUSCRIPT 511

EVALUATION OF FACTORS RELATED TO GROWTH OF RIFT VALLEY FEVER VIRUS IN SUSPENDED CELL CULTURES

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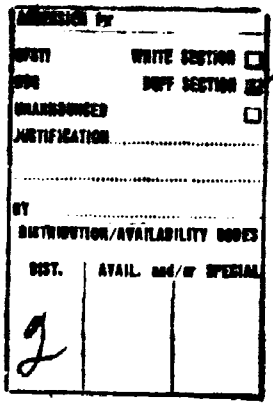

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ACKNOWLEDGMENTS

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ABSTRACT

The effect of several controlled variables on the peak titer and fold increase of Rift Valley fever virus grown in suspension culture on two variants of Earle L cell, L-DR and L-MA Clone 1-1, was studied. No significant amount of cell-associated virus was found at 24 hours, indicating a release of virus soon after its formation. Mild sonic treatment of the virus produced in serum-free medium increased the infective titer about 10-fold. This difference was not observed with virus produced in medium supplemented with serum. Peak titer is not affected by medium used during the infection period, multiplicity of inoculum (MOI), or initial cell concentration within the test range of 1×10^4 to 2×10^6 cells per ml. Cell strain employed influenced titer, because the L-DR cell did not produce virus efficiently at low MOI and low initial cell concentration. The time of peak titer and fold replication was dependent on MOI and initial cell concentration. Differences in virus propagation in monolayer and suspension systems are discussed.

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I. INTRODUCTION*

Rift Valley fever virus (RVFV) is an arthropod-borne virus prevalent in the Kenya area of Africa. It is a disease that infects domestic animals as well as man. The first successful cultivation of RVFV in tissue culture was accomplished almost 37 years ago by Daubney, Hudson, and Garnham,[1] using blood plasma or serum from infected mice as a source of cells. Saddington[2] was the first to propagate RVFV successfully on a chorioallantoic membrane of 9- to 10-day-old chick embryo. The virus was present in large numbers on the membrane, amniotic fluid, and livers from the embryos 5 days post-inoculation. It was also successfully propagated in the yolk sac and the chorioallantoic membrane by Kaschula[3] who obtained highest titers of the virus from 8-day-old embryonic eggs inoculated in the yolk sac and harvested 48 hours post-inoculation.

RVFV has also been grown in several types of tissue cell cultures.[4-11] In addition, both the neurotropic and pantropic variants of the RVFV were titrated successfully, using plaque formation on rat sarcoma cells,[9] Chang's human liver cells,[12] and sheep kidney cells.[13] Randall et al.[14] used monkey kidney cells to produce a vaccine to RVFV.

Orlando, Delauter, and Riley[15] reported that the optimum virus input multiplicity was approximately 2.5 $MICLD_{50}$. More recently, Johnson and Orlando,[16] using a monolayer tissue culture system of mouse fibroblast-like cells, described the growth of RVFV and reported on optimum pH, input multiplicities, and medium volume for this system. They also developed and described growth curves for both monolayer and suspension systems.

At present, little information is available for optimizing conditions for the infection and propagation of RVFV in a suspension system. It was our purpose, therefore, to describe the growth of RVFV in suspension culture of two established tissue cell lines and to test the variables of multiplicity of inoculum (MOI), cell concentration, and tissue cell medium during infection. These variables were so arranged in factorial design that both main effects and interactions could be determined. On the basis of the data obtained, efforts were made to select a tissue cell line for optimizing growth conditions.

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II. MATERIALS AND METHODS

A. VIRAL STRAIN

A small plaque variant of the pantropic van Wyk strain of RVFV isolated by Boyle[17] and grown in cultures of L cells was used as the primary seed stock. The working virus stock was developed by two passages from the original isolation. The virus suspension containing 10^7 and 10^8 mouse intracerebral lethal doses ($MICLD_{50}$) was stored in 2-ml amounts at -175 C.

B. VIRAL ASSAY PROCEDURES

Suspension fluid from virus-infected culture was removed and 10-fold serial dilutions were made in a mixture of one part medium 199 and two parts of Hanks balanced salt solution (v/v) supplemented with 10% calf serum. White mice of the Fort Detrick Swiss-Webster strain weighing 10 to 14 grams were inoculated intracerebrally with 0.03 ml of virus dilution. Eight mice were inoculated per dilution. Deaths were recorded during the next 6 days, eliminating deaths that occurred during the first 24 hours, which were assumed to be traumatic. The probit method[18] of calculating $MICLD_{50}$ values was used.

C. DISRUPTION OF CELLS BY SONIC TREATMENT

The Branson Sonifier was used with 5.0 ml of cell suspension placed in a 12 by 75 mm Falcon plastic tube. These were cooled in tap water. The sonic oscillator probe was submersed in the cell suspension and, except as noted, the sonic treatment given was for 20 sec per 20 kc at an input of 10 watts. More than 99% of the cells were disrupted by this treatment.

D. MULTIPLICITY OF INOCULUM

MOIs were determined by the following formula:

$$\frac{A \times B \times C}{D} = \text{volume of virus inoculum to be used}^{19}$$

where

A is the tissue cell count per ml
B is the volume of tissue cells
C is the MOI based on $MICLD_{50}$ desired
D is the virus titer ($MICLD_{50}$/ml).

E. TISSUE CELL STRAIN

A variant of the Earle's L cell (L-MA) was obtained from Dr. Donald Merchant, University of Michigan, Ann Arbor, Michigan. Cells were maintained antibiotic-free. A selected clonal line designated L-MA Clone 1-1 was established and used as one of the tissue lines in this study. A second variant of Earle's L cell was also used, the L-DR line isolated and described by Daniels et al.[30]

F. TISSUE CULTURE PROCEDURE

The Merchant spinner flask* was used to grow tissue cells. All cultures were grown in antibiotic-free medium. The growth medium for the L-MA C1 1-1 cell was medium 199 supplemented with 0.05% Bacto-peptone. Medium used to propagate the L-DR tissue cell line was composed of Eagle's minimum essential medium (EMEM) as modified by Daniels et al.[30] Medium was supplemented with 10% bovine serum as noted. Viability of cells was determined by the erythrosin B exclusion method, and all cultures used had a viability of 95% or greater. Cells were free of contamination by pleuropneumonia-like organisms (PPLO), as determined by repeated failure to detect organisms on PPLO agar. Bacterial contamination was checked by suitable agar and suspension culture techniques. Throughout these studies, the tissue cells were routinely harvested near the peak of the log phase.

G. INFECTION OF CULTURES

Prior to infection, tissue cells were concentrated by centrifugation (3,000 rpm for 10 min at 4 C) and resuspended to the desired cell concentration in the test medium. Cultures were adjusted to pH 7.4 prior to infection. The desired MOI of the virus was seeded directly into suspension tissue culture flasks[21] and incubated on the reciprocal shaker (100 three-inch strokes/min) at 37 C for various periods of time, dependent on experimental design. The cultures were assayed for virus propagation at 0, 24, 48, 72 and 96 hours unless otherwise indicated by experimental design.

H. EXPERIMENTAL STUDIES

The work reported here is divided into four areas of investigation. In the first two preliminary experiments, the effect of sonic treatment of the culture and of cell concentration on the titer of the virus was determined. These experiments were followed by two factorial experiments for studying main effects and interactions of selected variables on infection and virus propagation in a suspension tissue culture system.

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* Quality Glass Apparatus, Inc., 2821 South State St., Ann Arbor, Mich.

Under certain conditions, RVFV tended to clump or aggregate, making sonic disruption necessary for an accurate assay. For this experiment, L-MA C1 1-1 tissue cells, grown either with or without serum supplementation of the medium, were employed. MOI was 0.01, cell concentration was 6×10^5/ml, and the infection period was 24 hours. The sonic input setting was 10 watts for 1 min. After this manipulation, virus suspensions were assayed in mice and $MICLD_{50}$ were determined. The difference between the test and the controlled $MICLD_{50}$ for determining both the effect of clumping on virus titer and the amount of cell-associated virus present was used for analysis of variance.

The second preliminary experiment to test the effect of RVFV on infectivity and virus propagation in the L-Ma C1 1-1 tissue cell line was propagated in peptone medium 199 and supplemented with 10% bovine serum. Tissue cell concentrations of 2×10^5, 1×10^6, and 2×10^6 were used. MOI was 0.01. Viral titers were assayed after 0, 24, 48 and 72 hours of incubation. $MICLD_{50}$ were calculated and incorporated into analysis of variance to determine the effect of cell concentration on viral infectivity and the possible interactions. Growth curves of RVFV at different cell concentrations are presented to illustrate different peak titers and when they occur in a suspension culture system.

The first major experiment was a Latin square designed to measure main effects and interactions in a suspension tissue culture infected with virus. Variables tested in this design were (i) five cell concentrations, (ii) five multiplicities of inoculum, and (iii) five media in which the tissue cells were suspended at the time of inoculation. (The number of levels for each of these main effects was the same, thereby constituting a Latin square design experiment.) A schema illustrating these variables and experimental design is shown in Table 1. This design was repeated with two tissue cell lines. $MICLD_{50}$ were calculated after assay at 0, 24, 48, 72, and 96 hours of viral replication or propagation. For optimization of virus titer, an analysis of variance was performed on the peak titers and the initial titers after inoculation. Both main effects and interactions were calculated, and growth curves were plotted to illustrate the various phases of viral replication or growth.

The optimum conditions for viral replication or propagation were determined from these results and incorporated into an unreplicated factorial arrangement evaluating cell line, infecting medium, cell concentration, and MOI. A schema illustrating both variables and design of experiment is presented in Table 2. $MICLD_{50}$ were determined after 0, 24, 48, and 72 hours to indicate the virus titer. An analysis of variance was performed to determine main effects and interactions. Growth curves were calculated after determining the main effects and interactions to indicate peak levels of RVFV infectivity or propagation.

TABLE 1. SCHEMA OF VARIABLES TESTED IN LATIN SQUARE DESIGN APPLICABLE TO CELL LINES L-MA C1 1-1 AND L-DR

Multiplicity of Inoculum	Tissue Cell Concentration per ml				
	2×10^6	2×10^5	1×10^5	2×10^4	1×10^6
1.0	M_1 [a]	M_2 [b]	M_3 [c]	M_4 [d]	M_5 [e]
0.1	M_5	M_1	M_2	M_3	M_4
0.0001	M_4	M_5	M_1	M_2	M_3
0.01	M_3	M_4	M_5	M_1	M_2
0.001	M_2	M_3	M_4	M_5	M_1

a. M_1 EMEM BS_{10}: Eagle's modified essential medium supplement with 10% bovine serum.

b. M_2 199 P BS_{10} medium: 199 peptone supplemented with 10% bovine serum - commercial powder w/o $NaHCO_3$; Grand Island Biological Co., Grand Island, N.Y. - Bacto peptone 0.05%: bovine serum.

c. M_3 USA 1 medium: Institute of Tissue Culture, Powdered Medium w/o $NaHCO_3$; Grand Island Biologic Co., Grand Island, N.Y.

d. M_4 La Ye medium: Lactalbumin hydrolysate yeast extract 10X Hanks BSS, 100 ml; dextrose, 1 g; lactalbumin hydrolysate, 5 g; yeast extract, 1 g; $NaHCO_3$, 1 g. Bring volume to 1,000 ml, sterilize by filtration.

e. M_5 199 P medium: 199 peptone commercial powder w/o $NaHCO_3$, Grand Island Biological Co., Grand Island, N.Y.

TABLE 2. SCHEMA OF VARIABLES TESTED IN FACTORIAL EXPERIMENT

MOI[a]	L-DR			L-MA C1 1-1		
	M_2[b]	M_3	M_4	M_2	M_3	M_4
			2×10^5 cells/ml			
0.1	1[c]	2	3	4	5	6
0.01	7	8	9	10	11	12
			2×10^6 cells/ml			
0.1	13	14	15	16	17	18
0.01	19	20	21	22	23	24

a. Multiplicity of inoculum.
b. Medium formulation as shown in Table 1.
c. Shake-flask code number.

III. RESULTS

A. SONIC-DISPERSED PARTICLES OF RVFV IN SUSPENDED SYSTEM

Preliminary experiments utilizing the monolayer system of propagation indicated that RVFV was unstable in a serum-free medium. Therefore, it was necessary to investigate and determine if this was also true in a suspension system. RVFV was propagated in a suspension system, using the L-MA C1 1-1 tissue cell line grown in serum-containing and serum-free media inoculated at 0.01 MOI. Four treatments were compared: the tissue cell and virus suspension with and without sonic treatment (Table 3, Treatments 1 and 2), the centrifuged culture (i.e., devoid of cells) with sonic treatment (Table 3, Treatment 3), and the sonic-treated culture centrifuged and again sonic-treated (Table 3, Treatment 4). Two important conclusions were drawn: (i) virus aggregation was indicated because virus titers were increased by sonic treatment in serum-free but not in serum supplemented medium, and (ii) cell-associated virus was not indicated because the supernatant contained the same titer of RVFV, regardless of other treatment, whether the tissue cells were present or not. Subsequent experimentation statistically important and, in effect, verified the treatment.

TABLE 3. TITER OF RIFT VALLEY FEVER VIRUS AS AFFECTED BY SONIC TREATMENT

	Log_{10} $MICLD_{50}$				
		199 Peptone with 10% Bovine Serum			
Treatment	Serum-free	Repl 1	Repl 2	Repl 3	Mean
1. Tissue cells + virus; not sonic-treated	5.9	5.0	6.0	5.9	5.6
2. Tissue cells + virus; sonic-treated	7.0	5.0	5.8	5.8	5.5
3. Tissue cells + virus; centrifuged,[a] solids removed, and supernatant then sonic-treated	7.0	5.2	5.7	5.7	5.5
4. Tissue cells + virus sonic-treated; centrifuged,[a] solids removed, and supernatant sonic-treated	6.8	5.5	6.2	6.0	5.9

a. Tissue cells were centrifuged at 3,000 rpm for 10 min.

The knowledge that only a small percentage of virus is cell-associated, along with the effects of sonification in a serum and serum-free medium, provides us with necessary information for accurate assay of virus infectivity and for further experimental design in optimizing infectivity and growth of RVFV in a suspension culture system. In all later work, sonic treatment for 20 sec at 10 watts was used for all virus suspensions.

B. EFFECT OF TISSUE CELL CONCENTRATION ON INFECTIVITY

Different cell concentrations infected with a MOI of 0.01 were assayed for peak titer after 0, 24, 48, and 72 hours after infection (Fig. 1). Statistical analysis of these data showed that cell concentration had no effect on titer at 72 hours; however, there was a significant difference between titers at the different assay times ($P = <0.01$). The 24- or 48-hour post-infection titers were 3 to 4 logs higher than the 0 time titer. Between 48 and 72 hours, the titers declined to approximately the same level; therefore, cell concentration within the range of 2×10^5 and 2×10^6 in a suspension system does not change the final titer of the culture. It is of interest that a 10,000-fold increase in viral titer was obtained with 2×10^5 cells per ml at 48 hours. Once it was known that final titer does not vary with cell concentration at 72 hours in a suspension system, it appeared desirable to determine the optimum interaction of cell lines, cell concentrations, and MOI in both serum and serum-free media.

C. LATIN SQUARE DESIGN

The Latin square experiment described in Section II of this report was used to test a large number of variables that could influence both virus infectivity and replication. The experiment was designed so that an orderly and systematic selection of variables required for optimum infectivity and virus replication could be incorporated into a formal factorial design for optimizing conditions. Statistical analysis of these data (Table 4) was performed on peak titers at 24, 48, 72, and 96 hours. The analysis showed (i) a probable difference between the two tissue cell lines, with L-MA Cl 1-1 having the higher virus yield ($P = <0.10$); (ii) a significant difference ($P = <0.01$) among the tissue cell concentrations; however, inconsistencies were observed, because 1×10^5 cell concentration gave the lowest peak titer, 2×10^6 and 2×10^5 produced the highest peak titers, and 1×10^6 and 2×10^4 were intermediate; (iii) medium and MOI had no effect on peak titer; and (iv) the interaction of tissue cell line with MOI was significant at the 95% level. This difference probably was caused by the low titer for the L-DR tissue cell at a MOI of 0.001 and 0.0001.

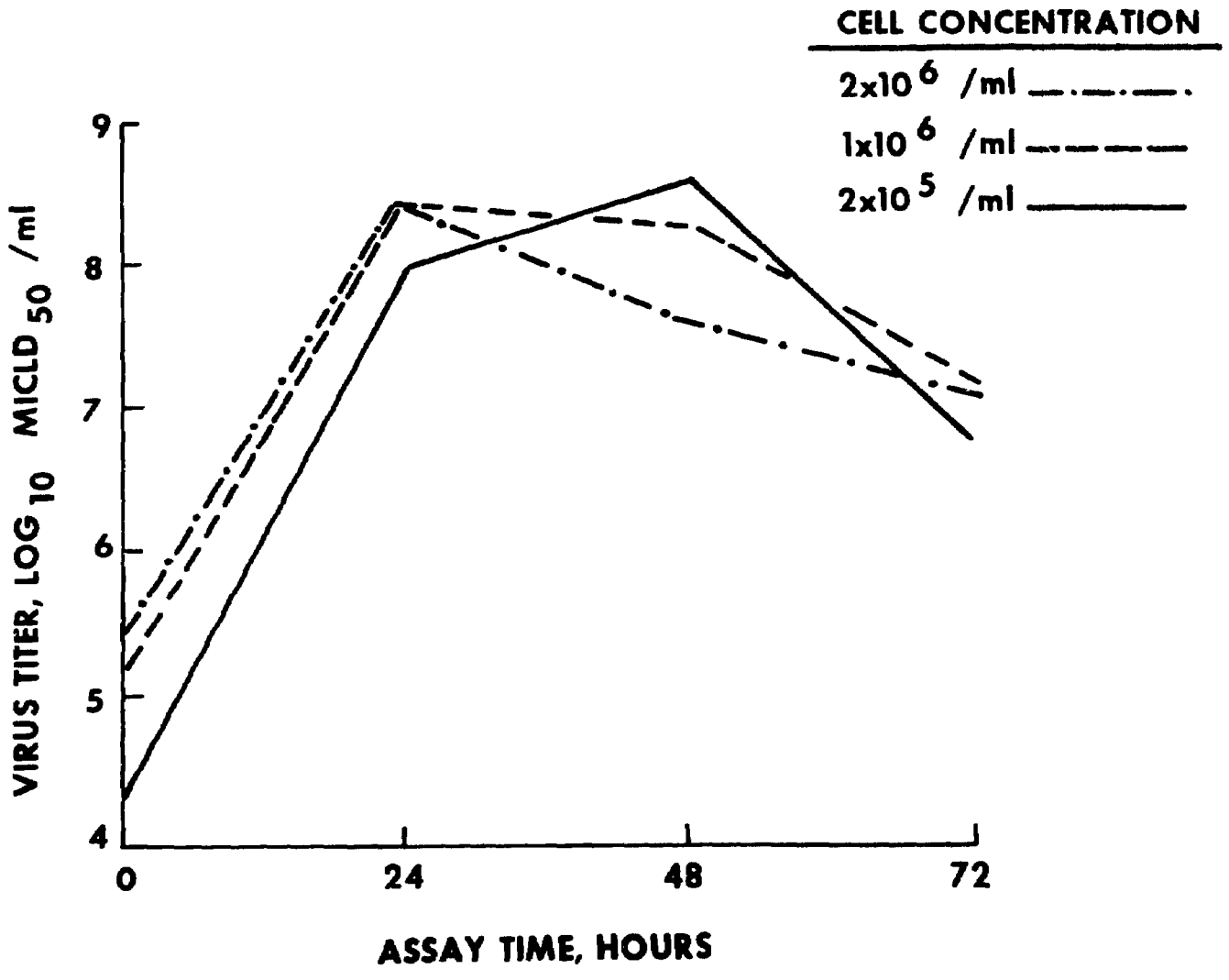


FIGURE 1. RVFV Titer Through 72 Hours for Three Cell Concentrations. Multiplicity of inoculum = 0.01.

TABLE 4. ANALYSIS OF VARIANCE FOR PEAK TITER OF RVFV, LATIN SQUARE DESIGN

Factor	df	Mean Square	F_0	Approx. Prob.
Cell Line (CL)	1	3.645000	2.91	<0.10
Cell Concentration (CC)	4	9.191875	7.35	<0.01
MOI	4	2.185625	1.75	NS
Medium (Med)	4	1.016875	<1	NS
CL x CC	4	0.248125	<1	NS
CL x MOI	4	4.648125	3.71	<0.05
CL x Med	4	1.548125	<1	NS
Error	24	1.251250		
TOTAL	49			

Analysis of variance was performed on the increase (log base 2 or fold increase) between initial and peak titers (Table 5) and showed (i) a significant difference between the tissue cell lines tested (P = <0.01), with L-MA Cl 1-1 producing the greater fold increase; (ii) a significant difference among the tissue cell concentrations; (iii) a significant difference among MOI (P = <0.01), apparently due to the low virus titer produced by MOI 0.001 and 0.0001 in the L-DR line; (iv) no significant difference among media, and (v) a significant interaction between cell lines and MOI at the 95% level. The results of this analysis on increase in virus were greatly influenced by the initial virus titer of the various treatment combinations, which ranged from <2.52 to 6.77 for all treatment combinations. Because type of medium was not significant, values for medium were combined, and the data are presented graphically (Fig. 2). Virus yield was plotted against assay time at different MOI and cell concentrations for the two tissue cell lines tested. The highest peak titer occurred for both cell lines at a tissue cell concentration of 1×10^6 per ml. MOI had no effect on the titer reached; however, the time to reach peak titer and the fold replication was dependent on MOI used. The only significant interaction was between cell line and MOI for both peak titer and fold replication of the virus (P = <0.005). The L-DR tissue cell line resulted in a much lower peak titer with low tissue cell concentration and low MOI. Since medium had no apparent effect, it appears that, as long as tissue cells are kept viable, virus replication proceeds independent of the type of medium employed. These observations were used to determine the variables tested in the factorial experiment next reported.

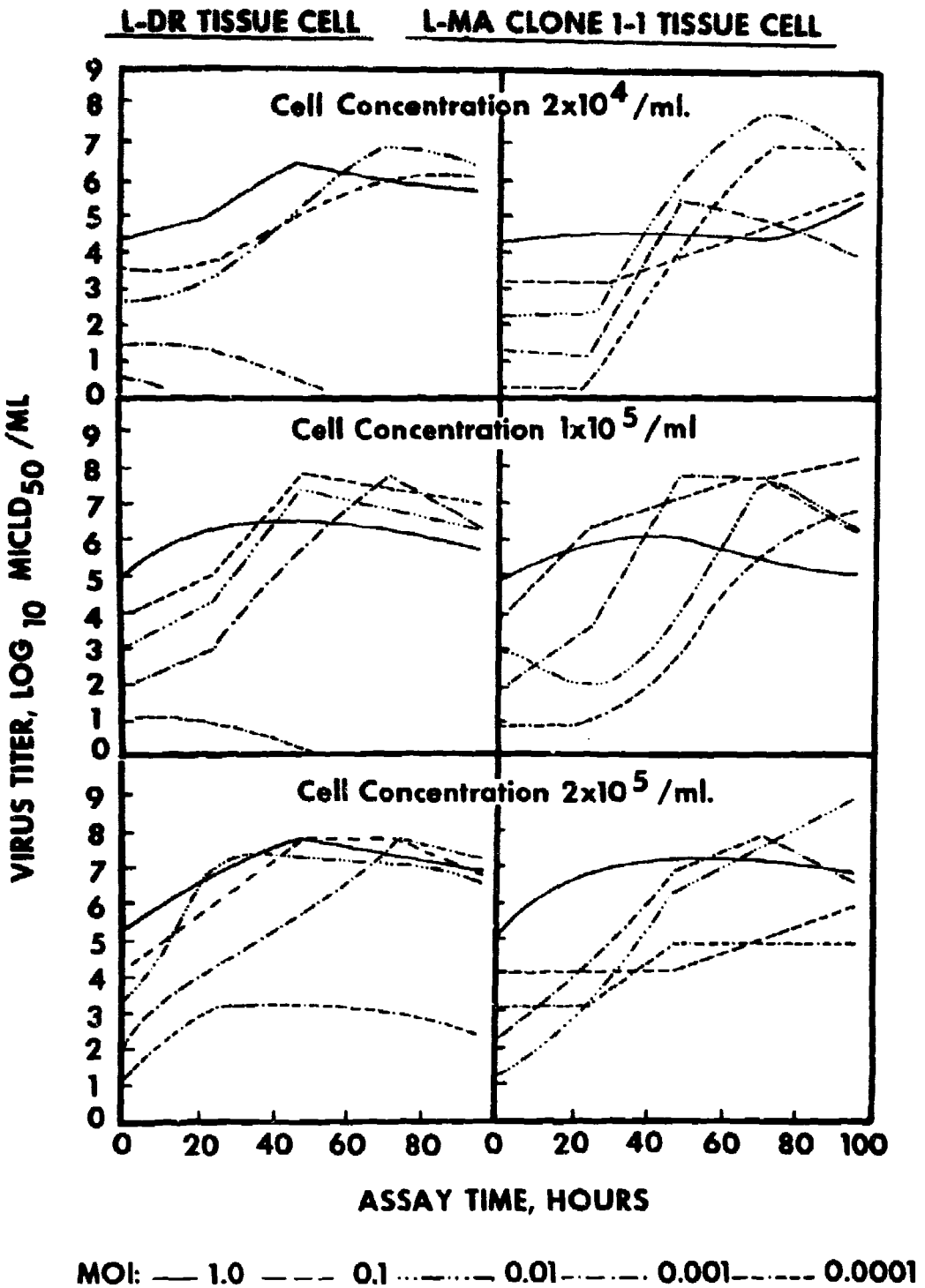
TABLE 5. ANALYSIS OF VARIANCE FOR INCREASE OF RVFV, LATIN SQUARE DESIGN

Factor	df	Mean Square	F_0	Approx. Prob.
Cell Line (CL)	1	10.242340	8.74	<0.01
Cell Concentration (CC)	4	1.504517	<1	NS
MOI	4	14.135447	12.06	<0.01
Medium (Med)	4	1.136287	<1	NS
CL x CC	4	0.517313	<1	NS
CL x MOI	4	3.789493	3.23	<0.05
CL x Med	4	1.465993	<1	NS
Error	24	1.171657		
TOTAL	49			

D. FACTORIAL DESIGN

From results obtained in the Latin square experiment, three different media were tested in two tissue cell lines at two different cell concentrations and at two MOI. This design placed emphasis on optimizing conditions for RVFV replication or growth in the two tissue cell lines under investigation. $MICLD_{50}$ were determined after 0, 24, 48, and 72 hours and incorporated into an analysis of variance. The results of this analysis (Table 6) showed (i) no significant difference between the two tissue cell lines tested because higher MOIs were used; (ii) a significant difference among the three media, with 199 medium supplemented with serum producing the highest peak titer ($P = <0.001$); (iii) a significant difference ($P = <0.01$) between tissue cell concentrations, with the 1×10^6 concentration producing the highest titer; (iv) a significant difference among the four assay time periods, with 48 hours post-infection producing the highest peak titer; (v) no significant differences among MOI; and (vi) significant interactions between (a) medium x cell concentration ($P = <0.05$), (b) MOI x time ($P = <0.05$), (c) medium x MOI x time ($P = <0.05$), and (d) MOI x cell concentration x time ($P = <0.01$).

From this analysis of variance, growth curves were plotted for each medium, combining tissue cell concentration and MOI (Fig. 3). In this factorial experiment, where significant variables had been selected and their critical level evaluated from the Latin square experiment, smaller differences were detectable. Medium containing serum was significantly better than medium without serum. (The serum possibly affected virus replication or stability on release from the cell.) MOI again was not statistically significant, with the higher MOI producing peak titers at earlier time periods than the low MOI. Time of peak titer also was affected by cell concentration. Tissue cell concentration again was highly significant Both tissue cell concentration and MOI affect the time of peak titer, and the concentration of both cells and MOI may be adjusted to effect the desired time of peak production.


L-DR TISSUE CELL
L-MA CLONE 1-1 TISSUE CELL
Cell Concentration 2x10⁴/ml.
Cell Concentration 1x10⁵/ml
Cell Concentration 2x10⁵/ml.
VIRUS TITER, LOG 10 MICLD50/ML
0 1 2 3 4 5 6 7 8 9
0 20 40 60 80 0 20 40 60 80 100
ASSAY TIME, HOURS
MOI: — 1.0 – – – 0.1 ·—·—· 0.01 -·-·- 0.001 -·-·-· 0.0001

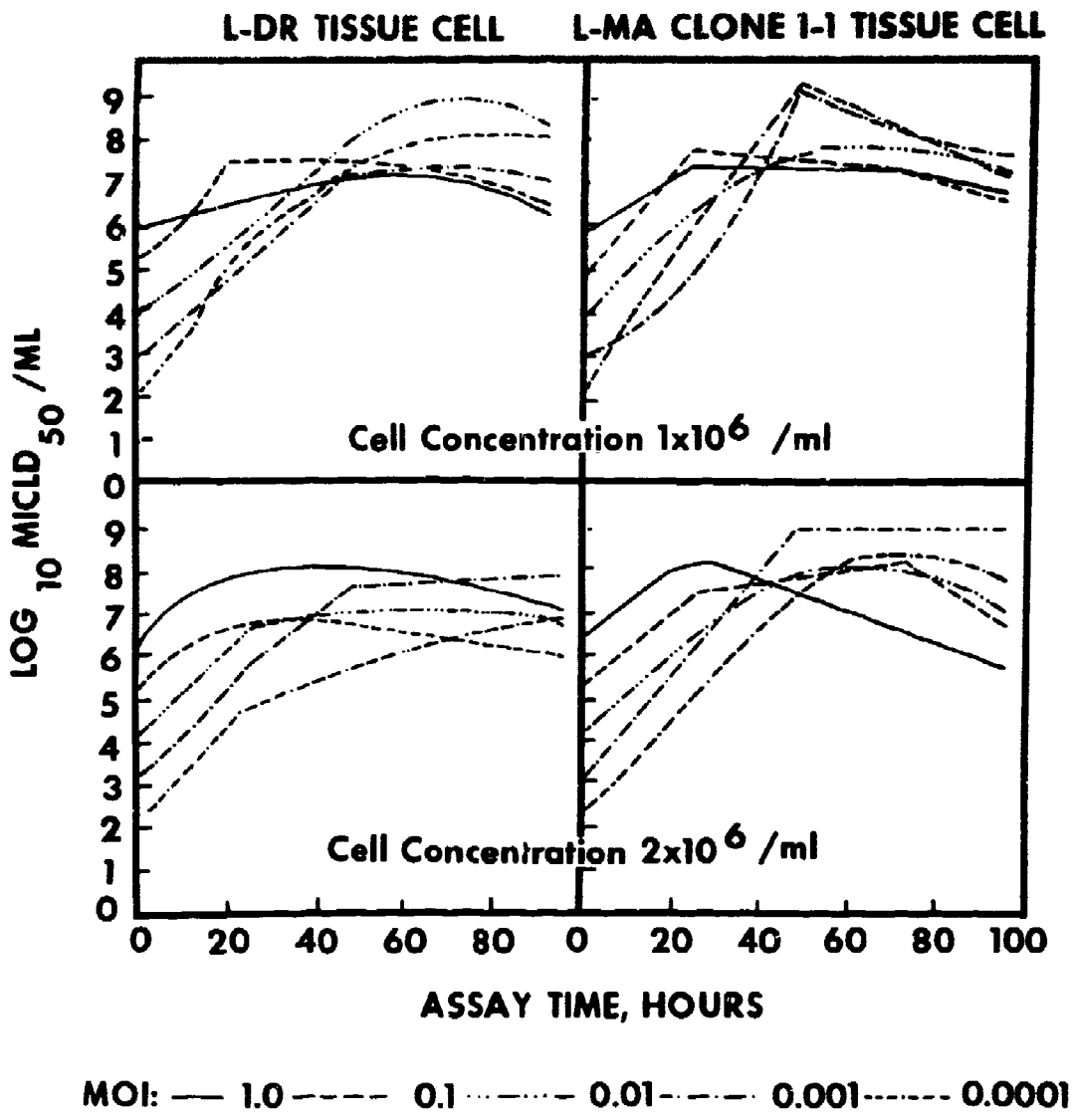


FIGURE 2. RVFV Curves Through 96 Hours in Two Cell Lines in Different Concentrations of Cells and Multiplicities of Inoculum; Latin Square Design.

TABLE 6. ANALYSIS OF VARIANCE FOR PEAK TITER OF RVFV, FACTORIAL DESIGN

Effect	df	Mean Square	F_O	Approx. Prob.
Strain (S)	1	2.9962667	3.65	NS
Medium (Med)	2	4.5100323	5.50	<0.01
MOI (O)	1	0.6370042	<1	NS
Concentration (C)	1	37.0016670	45.11	<0.01
Time (T)	3	52.103220	63.52	<0.01
Med x C	2	3.4973136	4.26	<0.05
O x T	3	3.5808627	4.36	<0.05
Med x O x T	6	2.0305427	2.47	<0.05
Pooled 4-way and 5-way interaction	29	0.8202286		
(All other interactions were not significant)				
TOTAL	95			

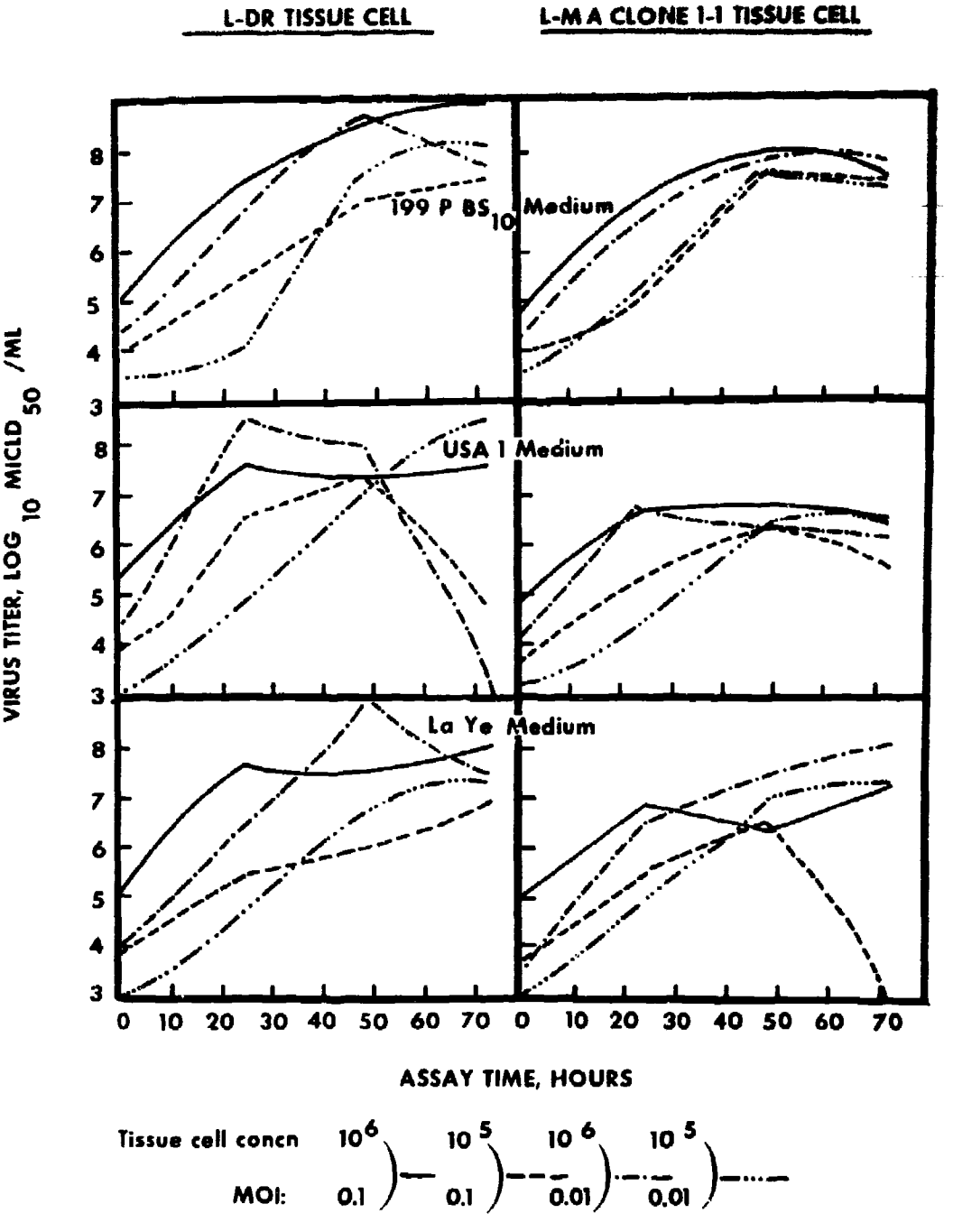


FIGURE 3. RVFV Growth Curves Through 72 Hours in Two Cell Lines in Different Media, Cell Concentrations, and Multiplicities of Inoculum; Factorial Design.

IV. DISCUSSION

Generally, the growth curve of RVFV in a suspension system is not unlike the general growth curves developed by Johnson and Orlando[16] for a monolayer system: A lag or latent period, a log or exponential growth phase, and a stationary phase preceding a death phase were observed. The time of initiation and length of each phase was dependent on MOI and cell concentration. The peak titer was not affected by any of the variables studied, indicating that inhibition or possibly inactivation of the virus takes place at approximately the same peak level of growth. The fact that the mean fold replication of virus varied by 2.8 logs but still reached the same peak titer supports the probability that viral replication stops because of inhibitory interaction between virus and tissue cells.

Significant differences between growth of RVFV in a suspension and in a monolayer culture are (i) the lack of cell-associated virus in suspension culture, as contrasted to the observations of Johnson and Orlando[16] for monolayer systems; (ii) the initiation of infection and growth to the same peak titer at very low multiplicity of inoculum as contrasted to the monolayer system.[16] We suggest that the ability of low MOI to reach the same peak titer may be attributable to the fact that cells and virus in suspensions are constantly in motion and consequently have multiple contacts and possibilities of cell attachment, but movement of virus in monolayer system is essentially Brownian motion.[19]

The lack of cell-associated virus in suspension culture indicates that the virus is released to the medium soon after its formation within the tissue cell. This observation contrasts with the findings of Johnson and Orlando,[16] but is supported by unreported work in our laboratories. Dr. L.E. Schneider* was unable to demonstrate virus aggregates within the cell by fluorescent RNA-specific staining techniques.

Increases in peak virus titer as high as 10-fold were attributable to sonic treatment of virus produced in serum-free medium, a difference that was not observed when RVFV was propagated in a serum-supplemented medium. Because inactivation of virus appears to be ruled out, the increase in titer resulting from sonic treatment most likely is attributable to changes in viral aggregate or molecular properties.

* Personal communication.

The L-DR cell did not produce virus when the MOI was 0.0001 with cell concentrations of 2×10^4, 1×10^5, and 2×10^5 or at an MOI of 0.001 with a cell concentration of 2×10^4; however, the L-MA Cl 1-1 line produced virus to the same titer as at higher MOI or cell concentrations. The L-DR cell was grown in serum-containing medium; the L-MA Cl 1-1 line was not. This difference in cell lines could be genetic; however, we believe it to be more likely a physiological or morphological change attributable to the serum, which affects either viral attachment sites or possibly attachment sites and replication.

From the growth curves established, and the great number of variables tested, it appears that optimum growth of RVFV in the suspension system is dependent on the tissue cell line only, irrespective of MOI, concentration of tissue cells, or medium employed during infection. Moreover, for optimum yields within a tissue cell line, the only limiting factor is the incubation time, which is entirely dependent on the MOI and concentration of cells used in a suspension tissue culture system. Melnick,[22] working with poliomyelitis virus, also noted that peak titer was not affected by inoculum size; however, the <u>time</u> at which the peak titer developed was dependent on the size of inoculum. It is also apparent from these observations that serum stabilizes the infectious virus particle over prolonged periods of incubation. Therefore, if serum-free medium is used as the infecting medium, then a high MOI and high cell concentration should be used to shorten the incubation time. It is apparent, too, that RVFV can be grown efficiently in a suspension system producing high yields of infectious RVFV particles. Such a system for other arboviruses seems directly applicable to producing high virus yields for vaccine production or to concentrating infectious RNA protein for chemical and genetic evaluation.

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13. ABSTRACT

The effect of several controlled variables on the peak titer and fold increase of Rift Valley fever virus grown in suspension culture on two variants of Earle L cell, L-DR and L-MA Clone 1-1, was studied. No significant amount of cell-associated virus was found at 24 hours, indicating a release of virus soon after its formation. Mild sonic treatment of the virus produced in serum-free medium increased the infective titer about 10-fold. This difference was not observed with virus produced in medium supplemented with serum. Peak titer is not affected by medium used during the infection period, multiplicity of inoculum (MOI), or initial cell concentration within the range test of 1 x 10⁵ to 2 x 10⁶ cells per ml. Cell strain employed influenced titer, because the L-DR cell did not produce virus efficiently at low MOI and low initial cell concentration. The time of peak titer and fold replication was dependent on MOI and initial cell concentration. Differences in virus propagation in monolayer and suspension systems are discussed.

14. Key Words

*Rift Valley fever
*Cultures (microbiology)
Growth
Growth curves
Suspension cultures
Sonic treatment
Titer
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